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Award Number: W81XWH-11-1-0391

TITLE: Novel High-Throughput Drug Screening Platform for Chemotherapy-Induced Axonal Neuropathy

PRINCIPAL INVESTIGATOR: In Hong Yang

CONTRACTING ORGANIZATION: The Johns Hopkins University
Baltimore, MD, 21205

REPORT DATE: May 201H
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TYPE OF REPORT: Annual
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PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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REPORT DOCUMENTATION PAGE			Form Approved OMB No. 0704-0188		
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1. REPORT DATE T æ ÁœFH		2. REPORT TYPE Annual		3. DATES COVERED 1 May 201G- 30 Apr 201H	
4. TITLE AND SUBTITLE : Novel High-Throughput Drug Screening Platform for Chemotherapy-Induced axonal neuropathy		5a. CONTRACT NUMBER			
		5b. GRANT NUMBER W81XWH-11-1-0391			
		5c. PROGRAM ELEMENT NUMBER			
6. AUTHOR(S) In Hong Yang E-Mail: iyang3@jhmi.edu		5d. PROJECT NUMBER			
		5e. TASK NUMBER			
		5f. WORK UNIT NUMBER			
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) The Johns Hopkins University Baltimore, MD, 21205		8. PERFORMING ORGANIZATION REPORT NUMBER			
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012		10. SPONSOR/MONITOR'S ACRONYM(S)			
		11. SPONSOR/MONITOR'S REPORT NUMBER(S)			
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Á Ú ā @ āÁ^ çÁ^•ç{ ÁÁ } ~ ā Á Á@Á@{ [@ ā ^Á ~*•Á Á ~æŏŏŏ & Á ā} •ÉQÁ æœ æVæ /ŏŏ Á ŏœ•^Á^ç^ Á āŏ ŏŏ āŏŏ &{ { ŏŏ Á ~æŏŏŏ & Á ā} ŏŏ ā *Á@Á^æŏ ^} ÉQÁ ā Á Á^ā &Á@Á āŏ ŏŏ ā &āÁ ā^Áæŏ É ^Áœ^Áŏ^} ŏāāÁ Á ~*•Á [ç} ŏŏ Á^~ } [çŏŏ^Áŏ &@{ [@ ā ^Áā &āŏŏ} āÁ^~ } āŏœ•Á{ Á ā ~*•ŏŏ āŏæ•Éŏ ^} çÉœÁŏŏ[ŏŏ ā ŏŏÁŏŏ•ŏŏ^Á}*[ā *ÉV^Á āç^Áœœœœ•^Á ~*•ŏŏ ÁÁ [ç} ŏŏÁ ā ~*•Á &@{ [@ ā ^Áā &āŏŏ} āÁ^~ } œœ•É					
15. SUBJECT TERMS P[]^Á çā^āÉ					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (include area code)
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Introduction:

Taxol is an antineoplastic agent, which is used for the treatment of various cancer types such as breast, ovarian, lung, bladder, etc. It disrupts the microtubule assembly and accumulates in the dorsal root ganglion (DRG). The side effect of Taxol is peripheral neuropathy. DRG neurons are sensory nerve cells, and their cell bodies are bundled together lying outside of the spinal cord - ganglia. DRG neurons are accessible and convenient to harvest in embryonic animals and they survive well in culture.

Results:

DRG from pregnant rats at 15th day of gestation were aseptically dissected and treated with collagenase and trypsin, mechanically dispersed by trituration and filtered to make a cell suspension. The cell suspension is diluted with Neurobasal Medium containing GDNF and B-27 supplement. The dissociated neurons are loaded at an appropriate density, to 96 well tissue culture plates that are coated with first with PDL and then with Laminin. The amount of neurons are 5000 to 8000 cells per well. The DRG cells are incubated in humidified 37 °C, 5% CO₂ incubator and their neurite growth and differentiation is visible under inverted light microscope after 24 hours of culture.

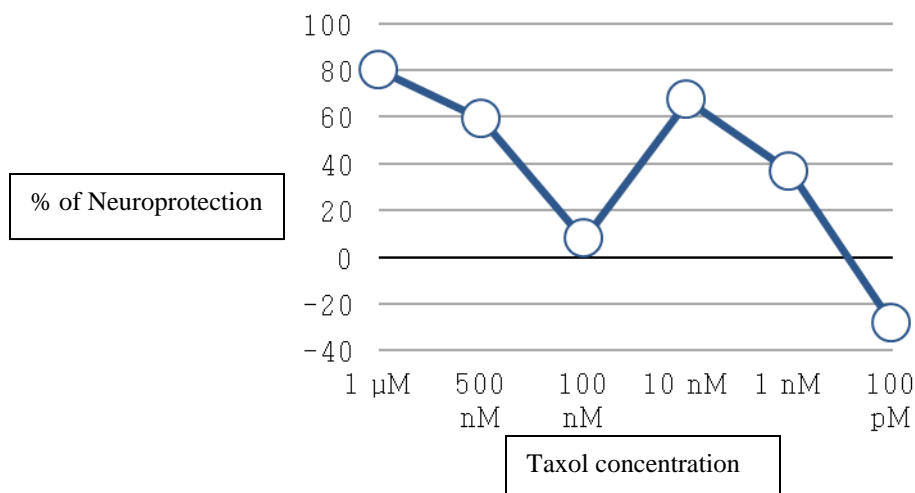
100 nMTaxol is added, in order to analyze the cytotoxic action of Taxol. Taxol with a final concentration of 100 nM, and the screened drugs at a final concentration of 1 µM are added to the wells.

The cells are incubated for 24 hours and cytotoxicity and therapeutic effect of drugs together with taxol are evaluated by the ATP assay, which is based upon the bioluminescent measurement of ATP that is present in all metabolically active cells. Since cells need ATP to remain alive the assay can be used for the direct assessment of cell numbers and viability. The amount of light measured by the luminometer is directly proportional to the amount of living cells present each well.

Each drug is screened twice and their average cytotoxicity/protectivity is calculated.

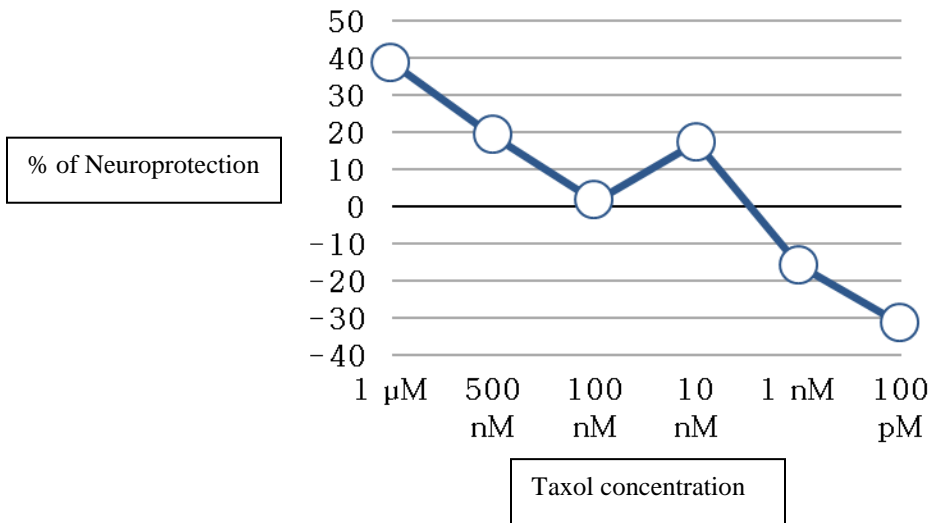
The percentage neuroprotection of the drugs are calculated according to formula:

$$\frac{[(\text{Viability of cells with drug+taxol}) - (\text{Viability of cells with taxol})]}{[(\text{Viability of cells in control}) - (\text{Viability of cells with taxol})]}$$

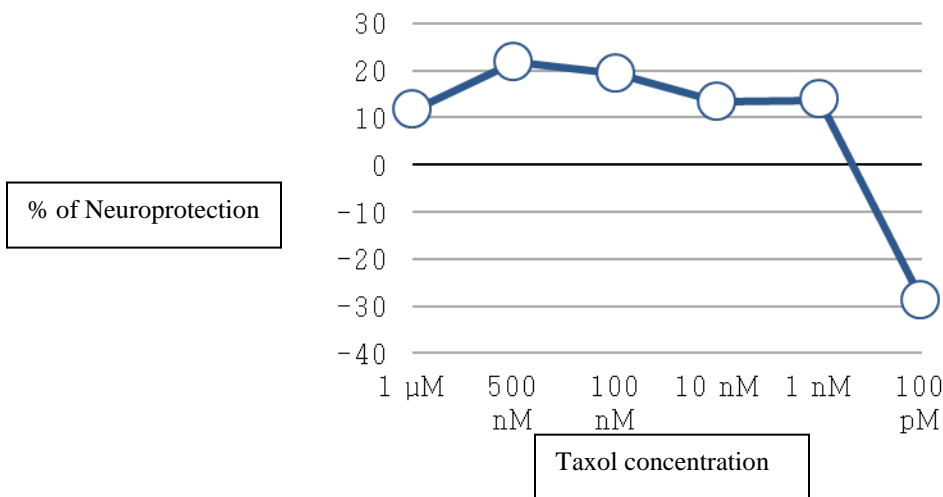


Drug Name: 1FM, Potential role: Antiinflammatory

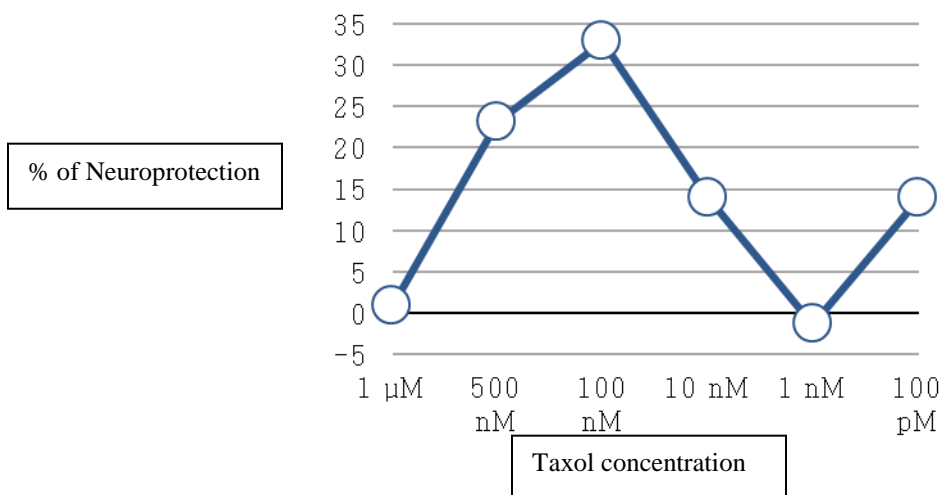
Chart 4



Drug name: 2FA, potential role: Anti-inflammatory.



Drug name: 4FA, potential role: Anti-Bacterial



Drug Name: 5HP, potential role: Anti-Bacterial

Key outcome from this research: From this research, we have identified 3 potential drugs for Taxol induced axonal neuropathies from 1470 drugs. Currently, we are testing the neuroprotective effect in Taxol neuropathy mouse model.

Conclusion: Taxol (paclitaxel and docetaxel) stabilize microtubules effectively treating various types of solid tumors. Through the disruption of microtubules of the mitotic spindle and the subsequent interference in axonal transport, Taxol induces toxic effect in peripheral neuron. From our drug screening, we have identified 4 potential drug candidates for Taxol neuropathies. Identified drugs are FDA approved drugs which readily is applied to clinical field. Identified drugs are 2 anti-inflammatory drugs and 2 anti-bacterial drugs. We don't know the neuroprotective mechanisms of identified drugs. The neuroprotective mechanisms of drugs, sites of actions, and in vivo testing of identified drugs are still on going. Also, the effect of identified drugs on tumors should be examined. We believe that the identified drugs will be beneficial to reduce the pain and discomfort in peripheral system of patients experiencing chemotherapy induced axonal neuropathies.